INVESTIGATION OF ANTIMICROBIAL ACTIVITY AND PHARMACOLOGICAL POTENTIAL OF BERBERINE

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

OF

SWAMI VIVEKANANDA UNIVERSITY

By

SURANJANA SARKAR

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF LIFE SCIENCES

SWAMI VIVEKANANDA UNIVERSITY

KOLKATA – 700121, WEST BENGAL, INDIA

2025

INVESTIGATION OF ANTIMICROBIAL ACTIVITY AND PHARMACOLOGICAL POTENTIAL OF BERBERINE

Submitted by

SURANJANA SARKAR

Registration Number: SVU/PhD/SCI/MB/002/2022

Under the supervision of

DR. PRIYAJIT BANERJEE

Assistant Professor

Department of Biotechnology

School of Life Sciences

Swami Vivekananda University

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY OF SWAMI VIVEKANANDA UNIVERSITY

2025

INTRODUCTION

Berberine is a prominent plant-derived isoquinoline alkaloid that has been used for generations across South and East Asia. In the Indian subcontinent, berberine-rich botanicals, particularly *Berberis* species, are embedded in long-standing medical systems including Ayurveda, Siddha, Unani, and regional folk practices, with traditional indications spanning gastrointestinal illness, febrile conditions, and infections. East Asian traditions, especially in China, similarly describe berberine-containing herbs for diarrhoeal illness and intestinal infections, and Traditional Chinese Medicine characterises these agents within frameworks used to treat dysentery and related mucosal conditions (Ai et al., 2021). Beyond this ethnomedical lineage, sustained scientific interest reflects berberine's broad biological profile, including reported antimicrobial, anti-inflammatory, and metabolic effects (Neag et al., 2018).

The accelerating rise of antimicrobial resistance (AMR) has renewed urgency around anti-infective discovery and optimisation, motivating systematic reassessment of historically used scaffolds under controlled conditions (Wang et al., 2025). In the antimicrobial context, however, berberine's development is constrained by pharmacological and pharmaceutic bottlenecks: its permanently cationic, planar scaffold is susceptible to a dissolution–permeability mismatch, transporter-mediated efflux, and extensive presystemic metabolism that together limit consistent exposure, while safety signals and context-dependent risk further narrow the development window (Torky et al., 2018). The literature has also operationally treated berberine hydrochloride as the default reference salt, despite the counter-ion being a non-neutral design variable that can influence dissolution behaviour, interfacial microenvironment, aggregation/precipitation tendencies, and tolerability, thereby confounding exposure-linked antibacterial readouts (Zhou et al., 2025). Against this backdrop, the present thesis adopted a formulation-engineering strategy, counter-ion substitution to a pharmaceutically acceptable citrate salt, evaluated in natural and sonication-derived

nanoforms, to generate head-to-head evidence on whether re-engineering chemical form and particle size can improve physicochemical performance and tolerability while preserving antibacterial activity under the same experimental framework.

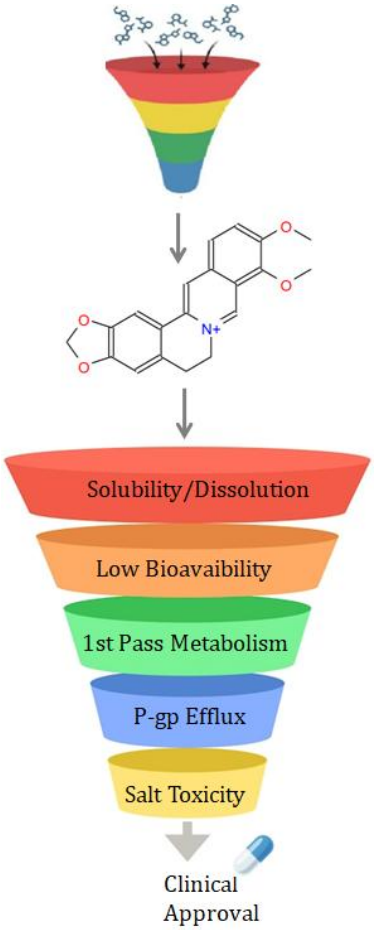

Figure 1 Funnel of translation for Berberine. Conceptual schematic illustrating the key biopharmaceutic and safety barriers that narrow berberine's progression toward clinical approval.

OBJECTIVES

This thesis conducted a formulation-level, head-to-head evaluation of four preparations, berberine hydrochloride (natural, nano) and a redesigned berberine citrate (natural, nano), to determine whether counter-ion substitution and sonication-enabled nanosizing modulate (i) physicochemical identity and key quality attributes alongside cellular and zebrafish toxicity readouts, (ii) antibacterial activity and MIC against *Staphylococcus aureus* with parallel

diffusion-based assessment against *Escherichia coli*, and (iii) broader pharmacological performance and translational constraints using supportive in silico analyses.

1. **Objective 1:** To Perform Integrated Physicochemical Characterization. and Biological Evaluation of Berberine Salts.

2. **Objective 2:** To Study Antibacterial Activity and MIC Determination of Berberine Salt.

3. **Objective 3:** To Assess the Pharmacological Performance and Translational Potential of Berberine.

MAJOR FINDINGS

Physicochemical profiling supported chemical integrity across salt forms and particle sizes. UV–visible scans (200–500 nm) showed the same characteristic absorption pattern for all formulations, with salt form and nanosizing primarily modulating intensity rather than producing substantive spectral shifts. FTIR resolved salt identity: citrate formulations displayed carbonyl and carboxylate-associated features (C=O stretch 1739–1702 cm^{-1}; COO^- bands ~1386/1343 cm^{-1}; C–O region 1276–1215 cm^{-1}) that were absent from hydrochloride, while shared bands across all samples indicated preservation of the berberine core. HPLC at 266 nm yielded a single, baseline-resolved berberine peak in each preparation, with retention times clustered around ~2.0 min and no meaningful impurities above detection limits. SEM confirmed a micrometre-to-sub-100-nm size shift following sonication in both salts.

Functional testing indicated that counter-ion identity and nanosizing altered dispersion, release behaviour, and toxicity signatures. Aqueous solubility measurements reported ~2.5 mg/mL for berberine hydrochloride (natural and nano) and ~7.5 mg/mL for natural berberine citrate at

neutral pH (25 °C), with nano-citrate described as showing markedly enhanced dispersion and apparent solubility; the qualitative observation of residual undissolved material in citrate dispersions was noted alongside these measured values. In HepG2 MTT assays (24 h; 1–25 μM), viability declined with concentration for both salts, with consistently lower viability in hydrochloride at ≥10 μM, supporting a milder cellular toxicity profile for citrate under the conditions tested. Zebrafish biochemical profiling differentiated salts: hydrochloride produced concentration-dependent increases in serum ALT/AST and inhibitory effects on muscle and brain acetylcholinesterase, whereas natural citrate showed only slight muscle acetylcholinesterase decline, brain acetylcholinesterase near control, and dose-related ALT/AST elevation (ALT crossing 200 IU/L) described as noticeably attenuated relative to hydrochloride. A dialysis diffusion model used as an exposure proxy showed the highest membrane transit for citrate (natural and nano), reaching ~18–19% cumulative release by 8 h, with nano-citrate maintaining a slight kinetic advantage over natural citrate.

Across agar well-diffusion assays, all formulations produced measurable inhibition against *Staphylococcus aureus,* whereas none produced satisfactory inhibition against *E. coli* under the same conditions. MIC modelling against *S. aureus* yielded 54.05 μg/mL (hydrochloride natural; 95% CI 37.20–85.50), 37.99 μg/mL (hydrochloride nano; 95% CI 27.92–54.09), 54.63 μg/mL (citrate natural; 95% CI 44.31–69.10), and 48.17 μg/mL (citrate nano; 95% CI 31.88–74.63); overlapping confidence intervals supported cautious interpretation of nano-enabled shifts as modest trends rather than large potency changes.

In silico analyses were used to contextualise, not replace, experimental phenotypes. Docking positioned berberine within the AcrB substrate-recognition region of the AcrAB–TolC efflux system with a favourable score (−9.5 kcal/mol), consistent with restricted accumulation as a plausible contributor to Gram-negative non-responsiveness. In Gram-positive context, docking to *S. aureus* FtsZ yielded a favourable energy (−7.8 kcal/mol), and MD simulations indicated

stability over a 50–60 ns trajectory with ligand RMSD stabilising between 0.35–0.65 nm; these findings were treated as mechanistic plausibility pending direct biochemical validation.

CONCLUSIONS

Taken together, the comparative dataset indicates that salt identity was a dominant determinant of formulation-level behaviour. Across orthogonal readouts, citrate substitution aligned with a consistently milder toxicity signature, higher HepG2 viability relative to hydrochloride at matched concentrations and an attenuated zebrafish biochemical perturbation pattern, including acetylcholinesterase effects and ALT/AST elevations described as lower than hydrochloride under comparable dosing. Functionally, the diffusion-based release proxy reinforced this directionality, with citrate (natural and nano) demonstrating higher membrane transit at 8 h than both hydrochloride preparations, and nano-citrate showing the most favourable kinetic profile within the tested set. Physicochemical verification by UV–visible, FTIR, HPLC and SEM supported that these differences were not attributable to loss of compound identity or major purity deviations, but were consistent with counter-ion–dependent dispersion and release behaviour that was further influenced by nanosizing.

On antibacterial performance, the work delineated a stable spectrum constraint. Anti-Staphylococcus aureus activity was retained across all preparations, whereas diffusion-based activity against *Escherichia coli* remained unsatisfactory irrespective of salt form or nanosizing. MIC estimates against *S. aureus* clustered within overlapping confidence intervals across salts and particle sizes, indicating that reformulation primarily reshaped tolerability and release proxies rather than producing a step-change in intrinsic antibacterial potency under the conditions tested. In silico modelling provided a coherent interpretive scaffold for these organism-level outcomes: favourable docking to AcrB supported efflux-mediated limitation as

a plausible contributor to Gram-negative non-responsiveness, while docking and MD stability with *S. aureus* FtsZ offered a mechanistic hypothesis compatible with Gram-positive susceptibility, explicitly remaining inferential without target-level validation.

Overall, the thesis establishes a formulation-development rationale in which counter-ion engineering (citrate) and nanosizing act as complementary levers: citrate substitution was associated with improved dispersion/solubility and enhanced membrane-transit behaviour alongside a comparatively attenuated toxicity footprint, while antibacterial action against *S. aureus* was maintained. At the same time, the persistence of gram-negative non-responsiveness across all formulations indicates that physicochemical optimisation alone may be insufficient to overcome permeability–efflux constraints, defining a boundary condition for translational expectations from salt and size manipulation in isolation.

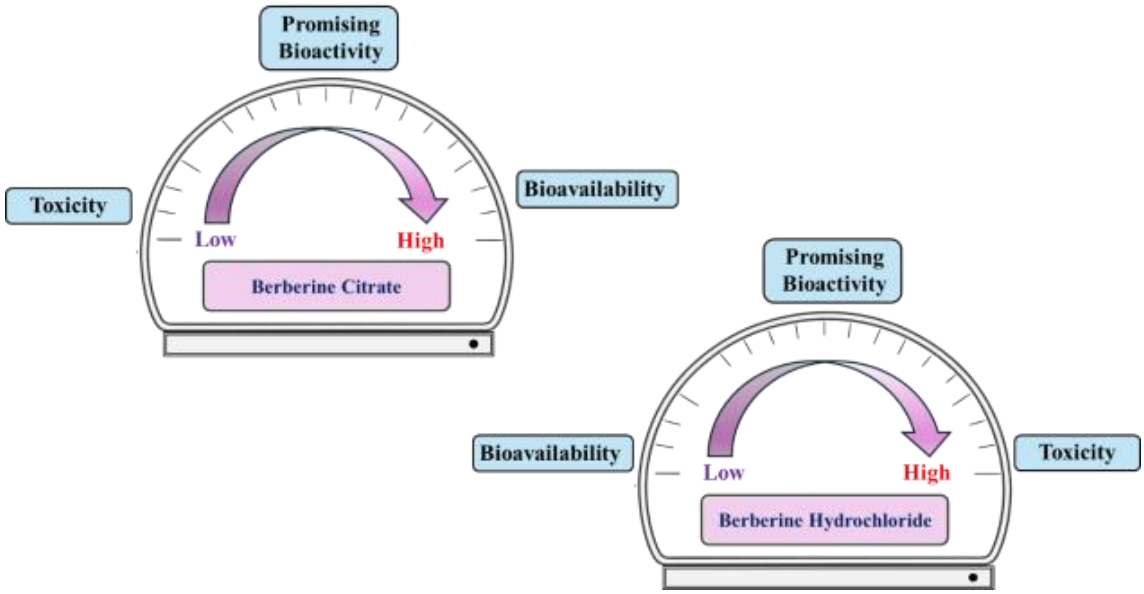

Figure 2 Model Depicting the Effect of Improved Berberine Salt Formulation in Therapeutic & Pharmacological Interventions.

References

Ai, X., Yu, P., Peng, L., Luo, L., Liu, J., Li, S., Lai, X., Luan, F., & Meng, X. (2021). Berberine: A Review of its Pharmacokinetics Properties and Therapeutic Potentials in Diverse Vascular Diseases. Frontiers in pharmacology, 12, 762654. https://doi.org/10.3389/fphar.2021.762654

M.A. Neag, A. Mocan, J. Echeverría, R.M. Pop, C.I. Bocsan, G. Crişan, A.D. Buzoianu, Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders, Frontiers in Pharmacology 9 (2018). https://doi.org/10.3389/fphar.2018.00557

Torky, A. S., Freag, M. S., Nasra, M. M. A., & Abdallah, O. Y. (2018). Novel skin penetrating berberine oleate complex capitalizing on hydrophobic ion pairing approach. *International journal of pharmaceutics*, *549*(1-2), 76–86. https://doi.org/10.1016/j.ijpharm.2018.07.051

Wang, L., Li, H., Wang, S., Liu, R., Wu, Z., Wang, C., Wang, Y., & Chen, M. (2014). Enhancing the antitumor activity of berberine hydrochloride by solid lipid nanoparticle encapsulation. AAPS PharmSciTech, 15(4), 834–844. https://doi.org/10.1208/s12249-014-0112-0

Zhou, F., Gu, X., Lin, M., Dai, Y., Wang, W., Xiong, Z., Li, Y., Jiang, M., & Wang, L. (2025). Mechanistic studies on the effect of berberine on methicillin-resistant Staphylococcus aureus drug resistance through modulation of wall teichoteic acid. Scientific reports, 15(1), 26003. https://doi.org/10.1038/s41598-025-11226-0